Increased carotid intima–media thickness is not associated with T-cell activation nor with cytomegalovirus in HIV-infected never-smoker patients

Tiphaine Goulenok[a], Anders Boyd[b], Martin Larsen[a], Solène Fastenackels[a], Franck Boccara[c,d,e], Jean-Luc Meynard[d,f], Nabila Hadour[c], Assia Samri[a], Moïse Desvarieux[g,h,i], Brigitte Autran[a,j], Victor Appay[a], Pierre-Marie Girard[b,d,f], Delphine Sauce[a], the CHIC Study group

Objectives: Increased risk of cardiovascular disease in patients infected with HIV has been attributed to immune activation, inflammation, and immunosenescence, all of which are linked to chronic immune activation by viral infections, particularly cytomegalovirus (CMV). Our aim is to evaluate the impact of these atherogenic markers in HIV-infected patients who never smoked.

Design: Exposure-matched, cross-sectional study.

Methods: In 59 HIV-infected individuals [$n = 30$ undergoing $\geq$4 years of antiretroviral therapy (ART); $n = 29$ never treated with ART] and 30 age-matched HIV-negative controls, we measured the level of activation and senescence, as well as the frequency of CMV-specific T cells, on peripheral blood mononuclear cells, while examining their association with carotid intima–media thickness. Partial correlations were adjusted for age, systolic blood pressure, and nadir $CD4^+$ cell count.

Results: The previously described roles of T-cell activation, CMV, and immunosenescence in the atherosclerotic risk of HIV-infected patients, as assessed by carotid intima–media thickness, were not apparent in our cohort of particularly 'healthy' HIV-infected never-smokers.

Conclusion: In HIV-infected individuals at low cardiovascular disease risk, our data show that the increased risk of carotid atherosclerosis is not associated with immunological markers described to be associated with HIV disease progression.

[a]INSERM U1135, UPMC UMRS CR7, Infections and Immunity, Université Pierre et Marie Curie- Paris 6, Hôpital Pitié Salpétrière, [b]INSERM, UMR_S1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, [c]AP-HP Department of Cardiology, Hôpital Saint-Antoine, [d]UPMC University Paris 06, [e]INSERM UMR_S938, [f]AP-HP Department of Infectious and Tropical Diseases, Hôpital Saint-Antoine, Paris, France, [g]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA, [h]INSERM U1153, Centre de recherche Epidémiologies et Biostatistique, [i]Ecole des Hautes Etudes en Santé Publique, and [j]AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Laboratoire d'immunologie Cellulaire et Tissulaire, Paris, France.

Correspondence to Delphine Sauce, INSERM U1135, UPMC UMRS CR7, Infections and Immunity, Hôpital Pitié Salpétrière, 91 bd de l'hôpital, 75013 Paris, France.

Tel: +33 1 40 77 98 89; e-mail: delphine.sauce@upmc.fr

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Introduction

Previous studies suggest that HIV-infected patients are at a high risk for both subclinical atherosclerotic disease and clinical cardiovascular disease (CVD) [1,2]. Several factors have been purportedly involved, such as higher frequency of 'traditional' cardiovascular factors [3] and antiretroviral therapy (ART)-containing protease inhibitors. More recently, immune activation and inflammation have emerged as important components in developing atherosclerotic lesions for both healthy persons and donors with chronic inflammatory diseases [4,5]. HIV-infected patients have been described to present with high levels of cytomegalovirus (CMV)-specific cells [6,7], which could play a role in the occurrence of non-AIDS inflammatory diseases. Indeed, Hsue *et al.* [8] demonstrated an association between CMV responsiveness and increased carotid intima–media thickness (c-IMT) in HIV-infected patients. Most recently, high CMV antibody titers were associated with several markers of subclinical atherosclerosis in ART-treated HIV-infected patients [9].

Previous studies conduced in HIV-infected individuals identify a number of parameters that could be involved in the development of atherosclerotic lesions. However, the heterogeneity of both the pathology and the examined cohorts (treated or not) renders difficult an overall conclusion on the key factors potentially responsible for the higher risk of CVD in such patients. Moreover, these studies have often been conducted *post hoc*, and in patients relatively advanced in HIV disease [8,9].

Here, we aimed at evaluating the impact of these previously described markers associated with atherogenesis (such as immune activation, CMV impact, and immunosenescence), in a particular homogenous group of young HIV-infected men who never smoked (never-smokers). These patients are known to be at a low risk of atherosclerosis.

Materials and methods

Study participants and samples

Participants were selected from The Collaboration on HIV, Inflammation and Cardiovascular disease (CHIC) study [10]. Briefly, 150 never-smoker men were enrolled in three groups: an index group of 50 HIV-infected patients above 35 years old, taking HAART for at least 4 years, and with HIV-1 viral load below 400 copies/ml; a second group of 50 individually age-matched (±5 years) patients, HIV-infected for at least 2 years, who were naive to ART; a third group of 50 HIV-negative patients individually matched by age (± 5 years) to the index HIV-infected, treated patient. All participants gave their written informed consent and the protocol was approved by the Hotel-Dieu Ethics Committee. Participants were excluded for any of the following: current or former smokers (>100 cigarettes/lifetime); history of coronary heart disease or stroke (including history of angina or myocardial infarction); active/chronic viral hepatitis infection; requiring current systemic chemotherapy or systemic steroids or HIV-2 infection.

In the present substudy, 90 patients (30 per group) were randomly selected from the initial study population. Peripheral blood mononuclear cells (PBMCs) (isolated by density gradient centrifugation) and sera were cryopreserved until use. One patient did not have available samples; thus only 89 were included in the present analysis. CMV serology was performed on serum samples using a Mastazyme-CMV serology kit (Mast Diagnostics, Merseyside, UK), according to the manufacturer's recommendations. Samples positive for CMV-specific antibodies were coined CMV-seropositive.

Assessment of carotid intima–media thickness

Carotid intima–media thickness scanning and reading used the INVEST protocol [11]. Briefly, c-IMT was calculated as a composite measure (mean of the 12 sites) that combined the near and the far wall of the maximal common carotid artery IMT, the maximal bifurcation IMT, and the maximal internal carotid artery IMT bilaterally, outside of plaque, as previously described [10]. c-IMT measurements were performed offline with IMT quality intima–media thickness (QIMT) automatic measurement software.

Reagents for flow cytometry

Directly conjugated and unconjugated antibodies were obtained from the following vendors: BD Biosciences (San Jose, California, USA): $CD4^+$ (APC-cyanin7), human leukocyte antigen-D-related (PE-Cy7), CD57 (fluorescein isothiocyanate), Ki67 (FITC), CCR7 (PE-Cy7), CD38 (APC), CD107a (Cy5-PE), interferon (IFN)γ (Alexa700), and tumor necrosis factor (TNF)α (PE-Cy7); Beckman Coulter (Villepinte, France): CD45RA (ECD); Caltag (Burlingame, California, USA): $CD8^+$ (Alexa405); Dako (Glostrup, Danmark): CD3 (Cascade Yellow); BioLegend (San Diego, California, USA): CD27

(AlexaFluor700). Cell surface marker staining was performed by addition of the respective antibodies for 15 min at room temperature. After incubation, cells were washed in phosphate-buffered saline and then permeabilized with Perm/fix kit (eBiosciences, San Diego, California, USA) before the addition of Ki67 antibody as previously described [12].

Cells were analysed on a LSR2 flow cytometer (Becton Dickinson, Franklin Lakes, New Jersey, USA) with appropriate isotype controls and colour compensation. Data were analysed using FlowJo v8.2 (Tree Star, Inc., Ashland, Oregon, USA) and DIVA software (BD Biosciences).

Polyfunctional assessment

Staining to assess functional capacity of CMV-specific T cells was performed after overnight stimulation of PBMCs with 15-amino acid long synthetic peptides overlapping by 10 amino acids and spanning the two CMV proteins, pp65 and IE1, as previously described [13]. Levels of CD107a, TNFα, IFNγ, interleukin (IL)-2, and macrophage inflammatory protein (MIP)-1β were analyzed with Pestle v1.6.1 and Spice v4.2.2 (Mario Roederer, Immuno Technology Section VRC/NIAID/NIH). Polyfunctionality was quantified with the polyfunctionality index algorithm [14], employing a beta version of the 'FunkyCells Boolean Dataminer' software (www.FunkyCells.com) provided by Dr Martin Larsen (INSERM U1135, Paris, France).

Statistical analysis

Data were compared between groups using the non-parametric Mann–Whitney *U* test. Pearson's correlations (or partial correlations) were used to compare cellular parameters with c-IMT levels, as appropriate. In previous

Table 1. Study population description of demographic and HIV characteristics.

	HIV-positive			
	Treated >4 years with ART group 1 ($n=30$)	Never treated with ART group 2 ($n=29$)	HIV− Group 3 ($n=30$)	P*
General characteristics				
Age, years mean (SD)	41.3 (5.9)	40.0 (7.6)	42.7 (6.6)	NS
Ethnic background [*n* (%)]				NS
European	19 (63.3)	19 (65.5)	21 (70.0)	
Sub-Saharan Africa	6 (20.0)	6.9 (2)	6.7 (2)	
French West Indies	0	3 (10.3)	0	
Northern Africa	4 (13.3)	1 (3.5)	3 (10.0)	
Other	1 (3.3)	4 (13.8)	4 (13.3)	
BMI [kg/m^2 mean (SD)]	23.8 (4.5)	23.8 (3.3)	24.3 (2.9)	NS
Diabetes [*n* (%)]	0	1 (3.5)	0	NS
Metabolic syndrome [*n* (%)]	3 (10.0)	4 (13.8)	3 (10.0)	NS
Hypertension [*n* (%)]	2 (6.7)	5 (17.2)	0	3
c-IMT [mm mean (SD)]	0.761 (0.078)	0.732 (0.083)	0.731 (0.057)	NS
Pro-inflammatory score [mean (SD)]	29.5 (3.0)	29.2 (5.3)	28.3 (2.9}	NS
Anti-inflammatory score [mean (SD)]	11.3 (2.4)	11.7 (1.7)	12.4 (2.8)	NS
Framingham 10-year risk score % [median (IQR)]	4.0 (2.0–6.0)	4.0 (2.0–4.0)	4.0 (2.0–4.0)	NS
D:A:D 10-year risk score % [median (IQR)]	3.1 (2.6–5.6)	2.5 (2.0–3.8)	3.0 (2.4–4.7)	NS
HIV infection				
Duration of HIV infection [years mean (SD)]	12.0 (4.9)	6.3 (4.9)	–	0.0001
Duration of cART [years mean (SD)]	8.8 (2.3)		–	
CD4$^+$ cell count [cells/μl mean (SD)]	585 (136)	454 (220)	717 (176)	1, 2.3
Nadir CD4$^+$ cell count [mean (SD)]	251 (163)	404 (166)	–	0.001
HIV-RNA[$\log_{10}$ copies/ml median (p25–75)]	1.60 (1.60–1.60)	4.12 (3.44–4.67)	–	0.0001
HIV-RNA <40 copies/ml [*n* (%)]	30 (100.0)	1 (3.4)	–	<0.001
Other infections				
Presence of CMV antibodies [*n* (%)]				
IgG	28 (93.3)	27 (93.1)	14 (46.7)	2.3
IgM ($N=68$)	0	2 (8.7)	1 (4.8)	NS
Presence of HCV antibodies [*n* (%)]	2 (6.7)	0	0	NS
Concomitant treatment				
Antihypertensive medication [*n* (%)]	3 (10.0)	4 (13.8)	0	NS
Beta-blockers [*n* (%)]	1 (3.3)	1 (3.5)	0	NS
Calcium channel blockers [*n* (%)]	0	1 (3.5)	0	NS
Renin–angiotensin antagonists [*n* (%)]	2 (6.7)	2 (6,9)	0	NS
Lipid-modifying agent [*n* (%)]	0	1 (3.5)	0	NS
Insulin or sulphonamode use [*n* (%)]	0	1 (3.5)	0	NS

cART, combination antiretroviral therapy; CMV, cytomegalovirus; D:A:D, Data collection on Adverse Effects of Anti-HIV Drugs; HCV, hepatitis C virus; IQR, inter-quartile range.

*Significance determined using a two-tailed *t* test or Kruskal–Wallis equality-of-populations rank test for continuous variables and Pearson chi-square test or Fisher's exact test for categorical variables. Significant differences were indicated as follows: 1, $P<0.05$ between groups 1 and 2; 2, $P<0.05$ between groups 1 and 3; 3, $P<0.05$ between groups 2 and 3; ns: no significant difference.

analysis [10], we adjusted c-IMT levels on age, hypertension, and diabetes (overall), and added nadir $CD4^+$ cell count, when including only HIV-infected patients. For the adjustments herein, prevalence of both hypertension and diabetes was very low in this study, resulting in the replacement of hypertension with SBP and removal of diabetes.

Statistical analysis was performed using GraphPad prism (v5.00; San Diego, California, USA) and STATA (v12.1; College Station, Texas, USA) software. *P* values less than 0.05 were considered significant.

Results

Demographic and patient characteristics

The CHIC study was designed to determine whether the increased risk in subclinical atherosclerosis observed in HIV-infected patients exists, independent of smoking; whether this increased risk correlated with HIV infection or ART; and whether the evaluation of pro-inflammatory/anti-inflammatory balance could illuminate the underlying subclinical mechanisms. The initial study showed that infection duration and inflammatory status are associated with atherosclerotic risk (defined as increased c-IMT) [10]. From the 150 initial participants, we have characterized the cellular immune system of 89 individuals distributed in the three infection/treatment groups, as represented in Table 1. Briefly, all groups had comparable ages, with no significant differences between the infection/treatment groups. Treated HIV-infected patients had significantly longer prevalent duration of known HIV infection than untreated patients (12 vs. 6 years, respectively; $P = 0.0001$). Among treated HIV-infected patients, HIV infection was well controlled with 100% achieving HIV viral load below 40 copies/ml and a median $CD4^+$ cell count of 585/μl. Overall, 69 (77.5%) patients were CMV-seropositive, with a significantly higher proportion in the HIV-infected groups vs. HIV-negative controls (93.2 vs. 46.7%; $P < 0.001$). Ten-year risk of any CVD event was low in this study population, whether determined by the Framingham risk score [median 4.0%, inter-quartile range (IQR) 2.0–6.0%) or Data collection on Adverse Effects of Anti-HIV Drugs (D:A:D) risk score (median 3.0%, IQR 2.2–4.3%). Accordingly, mean c-IMT was low overall (0.741 mm, SD = 0.074) [10]. Our analysis was stratified for treatment history and duration of known HIV infection [Supplementary Tables 1 (http://links.lww.com/QAD/A609) and 2 (http://links.lww.com/QAD/A610)].

Influence of T-cell activation on carotid intima–media thickness in HIV-untreated patients

$CD8^+$ T-cell activation (as measured by CD38 and Ki67 expression) among memory subset was significantly higher in HIV-infected patients compared to the uninfected controls (median % 1.17, IQR 0.54–2.32 vs. 0.55, IQR 0.28–0.91, respectively; $P = 0.002$) and was significantly correlated to HIV-RNA viral load ($r = 0.35$, $P = 0.007$). However, untreated HIV-infected individuals exhibited a significantly higher level of activation within the $CD8^+$ memory T-cell compartment (2.30%, IQR 0.36–1.03%) compared to treated HIV-infected (0.59%, IQR 0.36–1.03%; $P = 0.0001$) and HIV-negative patients (0.55%, IQR 0.28–0.91%; $P = 0.0001$) (Fig. 1a). The same significant differences were observed for $CD4^+$ T-cell activation (Fig. 1a), confirming previous studies [8,15]. Moreover, no correlation between the values of c-IMT and the level of activation within the $CD4^+$ T-cell compartment was observed in untreated HIV-infected individuals and not-treated HIV-infected patients ($r = 0.21$, $P = 0.12$; Fig. 1b after adjusting for age, SBP, and $CD4^+$ cell count). No association between c-IMT and the level of activation within $CD8^+$ was found ($r = 0.04$, $P = 0.8$).

Since our three groups of volunteers exhibited differences in their proliferative capacity ($CD38^+$ $Ki67^+$), we next examined the level of CD57 as a marker of turnover-related senescence. Our data showed that the frequency of senescent $CD4^+$, but not $CD8^+$, T cells was higher in HIV-seropositive individuals compared to HIV-negative controls (17.50%, IQR 9.31–28.90 vs. 5.72%, IQR 1.44–20.20%, respectively; $P = 0.004$) (Fig. 1c). However, there was no correlation between prevalent known HIV infection duration and senescent $CD4^+$ T cells ($r = -0.12$, $P = 0.4$). We also did not find an association between c-IMT and senescence in unadjusted analysis ($r = 0.02$, $P = 0.8$), as well as adjusted analysis overall (partial-*r* adjusted for age and SBP = 0.05, $P = 0.6$) or among HIV-infected patients (partial-*r* adjusted for age, SBP, and nadir $CD4^+$ cell count = −0.12, $P = 0.4$; Fig. 1d), as previously described by Kaplan *et al.* [16].

Subsequently, we decided to address the question of whether any of the measures reported to be associated with IMT in the initial study (e.g. anti-inflammatory profile) [10] could be associated with the immunological parameters described here. Looking at T-cell phenotype distribution, activation levels, senescence, and CMV responsiveness as immunological parameters, we found that only $CD4^+$ and $CD8^+$ T-cell activation was negatively correlated with duration of known HIV infection ($r = -0.42$, $P = 0.001$ and $r = -0.52$, $P < 0.0001$, respectively), but not with the pro-inflammatory or anti-inflammatory score.

Role of cytomegalovirus responses in increased carotid intima–media thickness in HIV-infected patients

Cytomegalovirus is frequently associated with inflammation [7,8,17]. We therefore characterized the frequency of CMV-specific cells among CMV-seropositive patients ($n = 69$) in order to investigate if CMV could be a

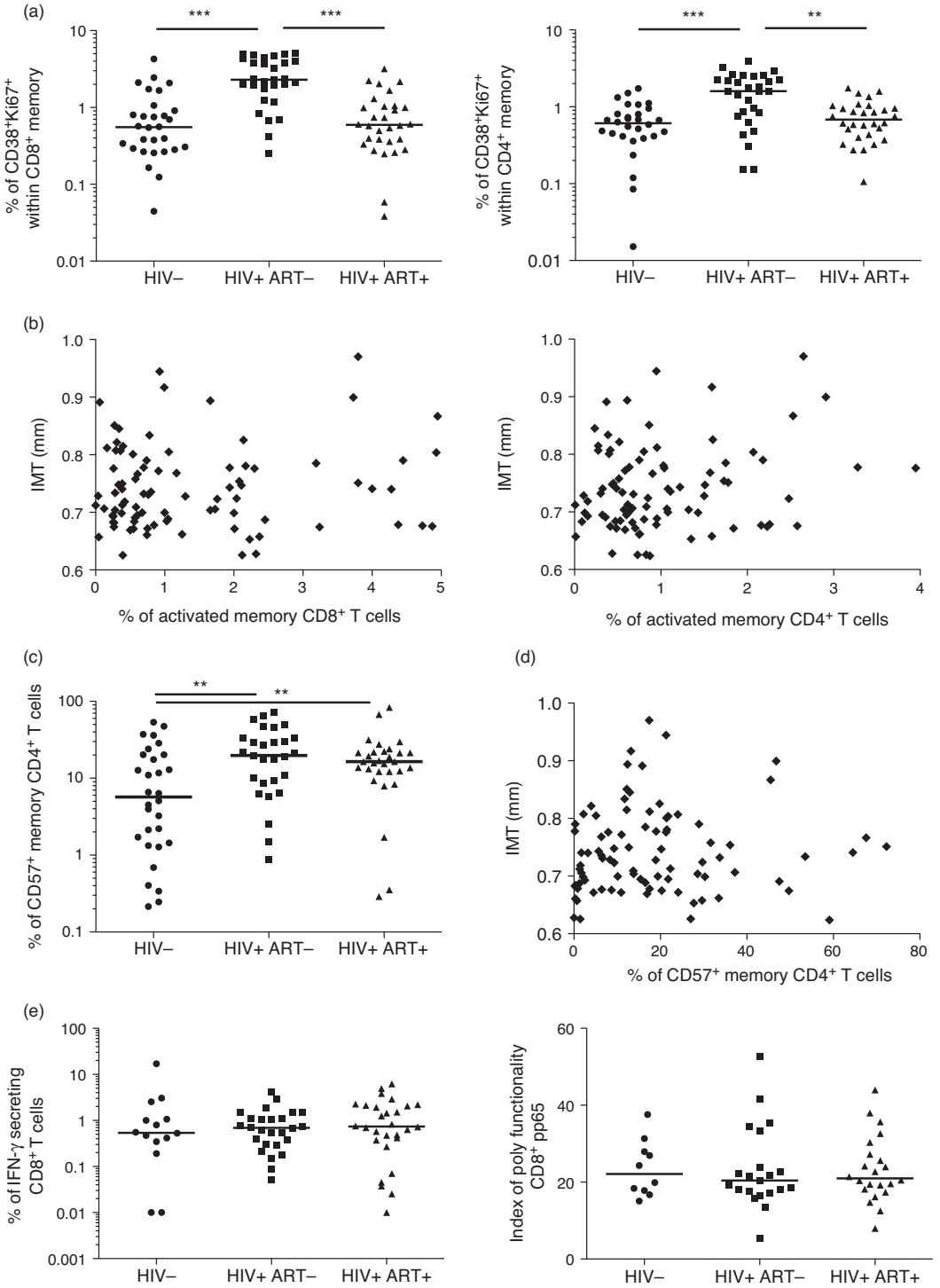

Fig. 1. Characteristics of HIV-seronegative vs. HIV-seropositive patients (black circles: HIV-seronegative donors; black squares: HIV-seropositive untreated patients; black diamond: HIV-seropositive, antiretroviral therapy-treated patients). (a) On the left and right panels, respectively: activated $CD8^+$ and $CD4^+$ memory T cells (CCR7-CD27-CD45RA+/–) were defined by the expression of CD38 and Ki67 markers. (b) Scatter plots showing the correlation between c-IMT values (nm) and the frequency of, on the left panel, activated $CD8^+$ T-cell memory or, on the right panel, activated $CD4^+$ T-cell memory. (c) Immunosenescence was defined by the expression of CD57 on memory T cells and was higher on $CD4^+$ T cells in HIV-seropositive patients compared to healthy controls. (d) Scatter plots showing the correlation between c-IMT values (nm) and the frequency of senescent $CD4^+$ T-cell memory. (e) CMV responsiveness assessed by the frequency of $CD8^+$ T cells secreting IFNγ (left panel) and by polyfunctionality index of $CD8^+$ T cells after overlapping pp65 peptides stimulation (right panel). (**) $P < 0.01$; (***) $P < 0.0001$. ART, antiretroviral therapy; c-IMT, carotid intima–media thickness; CMV, cytomegalovirus; IFN, interferon.

cofactor of increased c-IMT. Surprisingly, CMV-specific T-cell responses (as measured by the frequency of cells secreting IFNγ after stimulation with pp65 or IE1 overlapping peptides) were not significantly different in HIV-infected patients compared to the HIV-negative controls (0.72%, IQR 0.37–1.53 vs. 0.50%, IQR 0.19–1.01%, respectively; $P=0.3$; Fig. 1e). No correlation was also observed between prevalent duration of known HIV infection and anti-CMV $CD8^+$ T-cell response ($r=-0.05$, $P=0.7$). CMV-specific $CD8^+$ T-cell responses exhibit the same degree of polyfunctionality between all groups (based on the proportions of cells expressing combinations of IFNγ, TNFα, IL-2, MIP-1β, and CD107a [14] after stimulation with pp65 or IE1 peptides; Fig. 1e). Furthermore, there was no correlation with $CD8^+$ T-cell response and c-IMT levels in unadjusted analysis ($r=0.08$, $P=0.5$), as well as adjusted analysis overall (partial-r adjusted for age and SBP $=0.04$, $P=0.7$), or among HIV-infected patients (partial-r adjusted for age, SBP and nadir $CD4^+$ cell count $=0.09$, $P=0.5$).

Discussion

In the current study, we evaluated T-cell phenotype and CMV responsiveness in a cohort of HIV-infected never-smoking patients in order to establish which immune parameters could be predictive of c-IMT. Of note, c-IMT differences in these analyses were minimal compared to other studies [8,16]. This can be explained by the fact that the studied population is composed exclusively of relatively young men who have no cardiovascular risk factors, in particular, no obesity and no smoking.

We show here that levels of c-IMT were not associated with T-cell activation, senescence, and CMV. The levels of these cellular markers did not appear to change with increasing prevalent duration of known HIV infection.

One important consideration in this study, when compared to others, is the low levels of CVD risk and never-smoking status. Indeed, HIV-infected patients (treated or untreated) matched the HIV-negative controls on immunological parameters (level of senescence in $CD8^+$ memory T cells, CMV responsiveness) and on a number of clinical factors (age, BMI, diabetes, metabolic syndrome, etc.). The strength of our study therefore relies on the homogeneity of the groups who do not exhibit any differences in Framingham nor D:A:D 10-year risk scores, supporting that our data can be extrapolated in a general (non-HIV-restricted) context. This also pinpoints that, when successful therapeutic follow-up is performed, recently HIV-infected patients are not more at atherosclerotic risk than their uninfected counterparts. In conclusion, the impact of previously described HIV-associated comorbidities may be less severe for 'well treated' HIV-seropositive individuals. Moreover, our data support that overall health status (e.g. obesity and smoking habits) is an important parameter for HIV disease pathology and comorbidity, as suggested by a recent study, showing that smoking impacts on the immune system and therefore could drive accelerated disease progression [18].

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Conflicts of interest

The authors have no conflicts of interest to report.

References

1. Currier JS, Lundgren JD, Carr A, Klein D, Sabin CA, Sax PE, *et al*. **Epidemiological evidence for cardiovascular disease in HIV-infected patients and relationship to highly active antiretroviral therapy.** *Circulation* 2008; **118**:e29–e35.
2. Boccara F, Lang S, Meuleman C, Ederhy S, Mary-Krause M, Costagliola D, *et al*. **HIV and coronary heart disease: time for a better understanding**. *J Am Coll Cardiol* 2013; **61**:511–523.
3. Palella FJ Jr, Phair JP. **Cardiovascular disease in HIV infection.** *Curr Opin HIV AIDS* 2011; **6**:266–271.
4. Hansson GK. **Inflammation, atherosclerosis, and coronary artery disease.** *N Engl J Med* 2005; **352**:1685–1695.
5. Andersson J, Libby P, Hansson GK. **Adaptive immunity and atherosclerosis.** *Clin Immunol* 2010; **134**:33–46.
6. Naeger DM, Martin JN, Sinclair E, Hunt PW, Bangsberg DR, Hecht F, *et al*. **Cytomegalovirus-specific T cells persist at very high levels during long-term antiretroviral treatment of HIV disease.** *PloS One* 2010; **5**:e8886.
7. Appay V, Fastenackels S, Katlama C, Ait-Mohand H, Schneider L, Guihot A, *et al*. **Old age and anticytomegalovirus immunity are associated with altered T-cell reconstitution in HIV-1-infected patients.** *AIDS* 2011; **25**:1813–1822.
8. Hsue PY, Hunt PW, Sinclair E, Bredt B, Franklin A, Killian M, *et al*. **Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses.** *AIDS* 2006; **20**:2275–2283.
9. Parrinello CM, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, *et al*. **Cytomegalovirus immunoglobulin G antibody is associated with subclinical carotid artery disease among HIV-infected women.** *J Infect Dis* 2012; **205**:1788–1796.

10. Desvarieux M, Boccara F, Meynard JL, Bastard JP, Mallat Z, Charbit B, *et al*. **Infection duration and inflammatory imbalance are associated with atherosclerotic risk in HIV-infected never-smokers independent of antiretroviral therapy.** *AIDS* 2013; **27**:2603–2614.
11. Desvarieux M, Demmer RT, Rundek T, Boden-Albala B, Jacobs DR Jr, Sacco RL, Papapanou PN. **Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST).** *Circulation* 2005; **111**:576–582.
12. Sauce D, Larsen M, Fastenackels S, Roux A, Gorochov G, Katlama C, *et al*. **Lymphopenia-driven homeostatic regulation of naive T cells in elderly and thymectomized young adults.** *J Immunol* 2012; **189**:5541–5548.
13. Sauce D, Larsen M, Fastenackels S, Duperrier A, Keller M, Grubeck-Loebenstein B, *et al*. **Evidence of premature immune aging in patients thymectomized during early childhood.** *J Clin Invest* 2009; **119**:3070–3078.
14. Larsen M, Sauce D, Arnaud L, Fastenackels S, Appay V, Gorochov G. **Evaluating cellular polyfunctionality with a novel polyfunctionality index.** *PloS One* 2012; **7**:e42403.
15. Sauce D, Larsen M, Fastenackels S, Pauchard M, Ait-Mohand H, Schneider L, *et al*. **HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis.** *Blood* 2011; **117**:5142–5151.
16. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, *et al*. **T cell activation predicts carotid artery stiffness among HIV-infected women.** *Atherosclerosis* 2011; **217**:207–213.
17. Sacre K, Hunt PW, Hsue PY, Maidji E, Martin JN, Deeks SG, *et al*. **A role for cytomegalovirus-specific CD4+CX3CR1+ T cells and cytomegalovirus-induced T-cell immunopathology in HIV-associated atherosclerosis.** *AIDS* 2012; **26**:805–814.
18. Valiathan R, Miguez MJ, Patel B, Arheart KL, Asthana D. **Tobacco smoking increases immune activation and impairs T-cell function in HIV infected patients on antiretrovirals: a cross-sectional pilot study**. *PloS One* 2014; **9**:e97698.